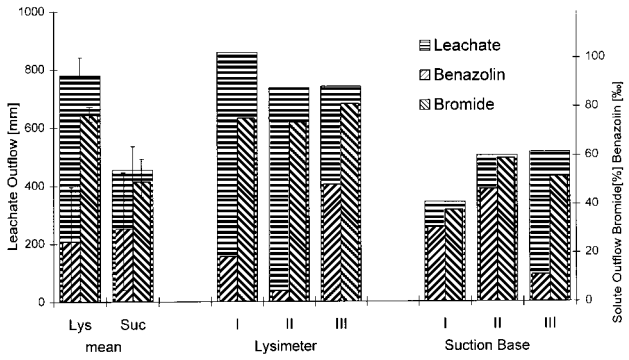

Figure 1. Cumulative leachate and solute outflow in the lysimeters and the suction bases after 2.5 years.

lower for the suction bases than for the lysimeters.

With respect to the six experimental plots of both systems, it was found that the bromide outflow revealed a high correlation with the leachate amount ($r = 0.94$); however, [^{14}C]benazolin outflow correlated neither with the drainage ($r = -0.18$) nor with the bromide outflow ($r = 0.05$).

It could be shown that the differences of the drainage (and the bromide) outflow were caused by different meteorological boundary conditions at the lysimeter and the field station which were *c*20 km apart. The average wind speed during the experiment was significantly higher at the field station than at the lysimeter station (1.5 m s^{-1} versus 0.9 m s^{-1}) as was also the average solar radiation (121 W m^{-2} versus 99 W m^{-2}). This can be explained by shading effects of the surrounding wire mesh cage and adjacent buildings.[1,2] When estimating the potential evapotranspiration with the Penman Equation, it was found that the evapotranspiration at the field station could be expected to be more than 23% higher than the lysimeter station.

Since no system-related differences between the lysimeters and the suction bases were found, it may be concluded that lysimeters were well-suited to examine the leaching behaviour of solutes in the sandy soil used for the experiments. Nevertheless, the variability of the [^{14}C]benazolin outflow demonstrated that it is not possible to assess the leaching tendency of a pesticide using only one lysimeter (of that given size). Several replicates are necessary to cover the variability of the physical and biochemical factors in the soil.

ACKNOWLEDGEMENTS

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Resistance to benzimidazole can be caused by changes in β-tubulin isoforms

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Abstract: The summary reports work that indicates that resistance in *Rhynchosporium secalis* to benzimidazole fungicides could result from substitution of the normal wild-type *benA* β-tubulin gene by other β-tubulin isoforms.

Keywords: resistance; *Rhynchosporium secalis*; benzimidazoles; β-tubulin isoforms

1 INTRODUCTION

Benzimidazole fungicides bind to β-tubulin and affect microtubule function. Field resistance to this group of fungicides in pathogen populations has been limited to mutations at codons 198 and 200 of the β-tubulin (*benA*) gene, although amino acid substitutions conferring benzimidazole resistance exist at another seven codon positions in laboratory-induced mutants.[1] In monitoring studies of field populations of the barley leaf scald pathogen *Rhynchosporium secalis* Davis we have identified two mutations linked to benzimidazole resistance. At codon 198 the change of **GAG** to **GGG** substitutes glycine for glutamic acid and at codon 200 the change of **TTC** to **TAC** substitutes tyrosine for phenylalanine. However, a point mutation generated in the laboratory substituting lysine for glutamic acid at codon 198 (**GAG** to **AAG**), has not been identified in the field and may carry a pathogenicity penalty.[2] As a first step to determine the effect of specific mutations on pathogenicity of *R secalis*, a wild-type benzimidazole-sensitive strain was transformed with an altered $\mathbf{GGG}_{198}$ gene.

2 EXPERIMENTAL

Strain 810 was transformed with vector pRSTUB3,1[3] by the protoplast method[4] and transformants selected on lima bean agar amended with the benzimidazole carbendazim (1 μg ml^{-1}). Two transformants, J2 and J3, were obtained and fungicide sensitivity levels assessed. In comparison to 810, both transformants have decreased sensitivity to carbendazim (Table 1), similar sensitivity levels to the *N*-phenylcarbamate dithiofencarb, but are at least 25-fold less sensitive to another carbamate, methyl *N*-(3,5-dichlorophenyl)carbamate (MDPC). Pathogenicity was determined by inoculating 20-day-

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Table 1. Minimum inhibitory concentration of fungicides ($\mu g\,ml^{-1}$)

	810	*J2*	*J3*
Carbendazim	0.4	2	2
Dithiofencarb	>50	>50	>50
MDPC	2	>50	>50

old barley plants (cv Tankard) with 20 µl of 1×10^{-6} spore suspension per leaf. Inoculated plants were left at 18°C with a photoperiod of 16 h; immediately after inoculation, humidity was increased by placing the plants in sealed plastic bags. After 72 h the bags were removed and the plants were maintained at 18°C for three weeks before assessment of scald symptoms.

Southern analyses of restricted DNA from J2, J3 and 810 probed with the *R secalis benA* β-tubulin indicated differences in the location of the β-tubulin gene (Fig 1), and suggested that the resident wild-type β-tubulin had been deleted in J2 and J3, neither of which showed similar banding patterns to the wild-type strain 810. When probed with linearised pUC18 a signal was evident only from J2, suggesting that J3 had not been transformed at all.

Two β-tubulin consensus primers B1 and B3[5] were used to obtain an 871 bp PCR product (from codon 134 to 414), which was purified and cloned into the pGEM-T Easy vector (Promega, Southampton, UK). Nucleotide sequence analysis of both the J2- and J3-derived products revealed that they were 98% identical to each other, but less identical to the published *R secalis* β-tubulin sequence[3] contained in vector pRSTUB3,1. The nucleotide sequence revealed that both J2 and J3 contained $\mathbf{GAG}_{198}$ and $\mathbf{TTC}_{200}$, which is the sequence associated with the wild-type benzimidazole-sensitive, phenylcarbamate-resistant field strains (Table 2).

At the amino acid level, J2 and J3 are 98% identical (codons 134 to 414), but are only 94.5% and 93% similar to the *R secalis* β-tubulin, respectively. None of the amino acid differences found between J2/J3 and the published *R secalis* sequence correlates with published amino acid mutations conferring resistance to benzimidazole fungicides.[1]

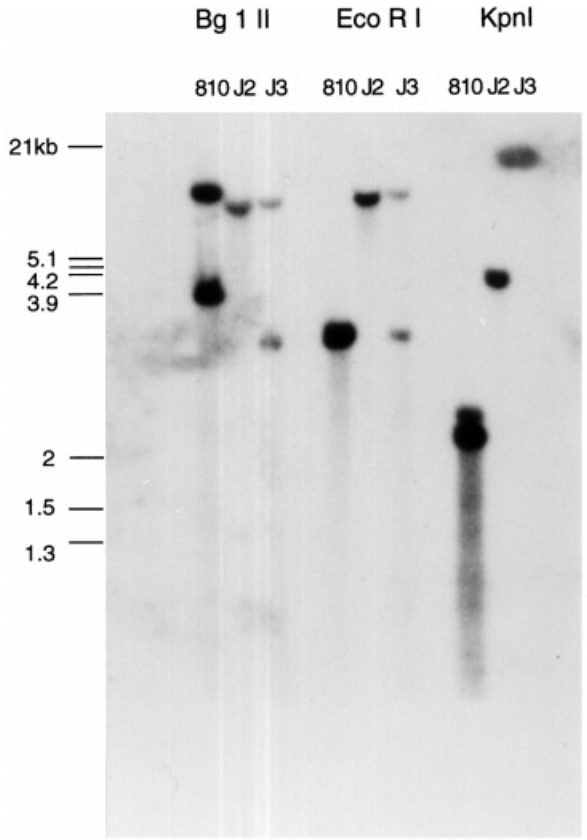

Figure 1. Hybridisation of the *Rhynchosporium secalis benA* β-tubulin gene to DNA from 810, J2 and J3. DNA digested with restriction endonucleases Bgl II, Eco RI and Kpn I.

3 DISCUSSION

These results indicate that benzimidazole resistance can be caused in *R secalis* through substitution of the normal wild-type *benA* β-tubulin gene by other β-tubulin isoforms. These two isoforms have the wild-type amino acid sequence at amino acid codons 196–202, and might be expected to bind benzimidazole fungicides.[6] What feature of the protein sequence affects fungicide binding is not clear because only partial amino acid sequences of these isoforms are available so far, but it does not include other aa changes within this region that are known to confer resistance.

Table 2. Amino acid sequence, fungicide sensitivity and pathogenicity in strains of *Rhynchosporium secalis*

Strain	*Nucleotide Sequence*						*Fungicide sensitivity*			
	198			*200*				*MBC*	*DFC*	*Pathogenic*
810	TCT	GAT	GAG	ACC	TTC	TGT	ATC	S	R	Yes
765	TCT	GAT	GGG	ACC	TTC	TGT	ATC	R	S	Yes
812	TCT	GAT	GAG	ACC	TAC	TGT	ATC	R	R	Yes
J2	TCT	GAT	GAG	ACC	TTC	TGT	ATC	S	R	Yes
J3	TCT	GAT	GAG	ACC	TTC	TGT	ATC	S	R	Yes

As J2 and J3 are pathogenic, there is potential for these isoforms to be present in field populations of *R secalis*. If so, oligonucleotide probes[3] designed to detect the benzimidazole-sensitive wild-type *benA* isoform would also hybridise to these other β-tubulin isoforms. To avoid false positives would require further PCR using primers specific for this isoform.

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